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ORIGINAL ARTICLE

A matter of history: effects of tourism on physiology, behaviour and breeding parameters in Magellanic Penguins (*Spheniscus magellanicus*) at two colonies in Argentina

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Abstract Increasing ecotourism activity in Argentine Patagonia has led to concerns about the effects of ecotourism on wildlife populations. Penguin breeding colonies are popular tourist destinations. While some species of penguins habituate to human visits, others exhibit negative effects due to disturbance. We studied the effects of tourism on Magellanic Penguins (*Spheniscus magellanicus*) at San Lorenzo colony located on the Peninsula Valdes, Chubut, Argentina. We measured behavioural, physiological, and reproductive parameters in birds nesting in tourist-visited and non-visited areas. San Lorenzo has 11 years of visitation history and a low intensity of visitors ($\sim$10,000 annually). After 15 min of a simulated tourist visit, penguins in the tourist area showed fewer alternate head turns and lower plasma levels of the stress hormone corticosterone than penguins in a non-visited area. However, penguins showed similar baseline levels of corticosterone between areas as well as similar levels of integrated corticosterone expressed after an acute stressor. Penguin breeding success and chick growth were similar between areas. We compared these findings to results previously published from Punta Tombo, a colony with a longer history (+50 years) and high intensity of annual visitors (>120,000 people). Many (reproductive and behavioural parameters), but not all (physiological parameters), of our findings are similar. That a physiological difference exists may suggest that the previous history of human visitation plays an important role in the response of the birds. Thus, the continuous monitoring of tourist activity is important, as a history of visitation disturbance seems to have an effect on how birds respond to tourists.

Keywords Behaviour · Breeding success · Corticosterone · Ecotourism · Growth · *Spheniscus magellanicus*

Zusammenfassung Steigender Ökotourismus im argentinischen Teil Patagoniens hat zu Bedenken über die Auswirkungen von Ökotourismus auf wildlebende Populationen geführt. Brutkolonien von Pinguinen sind beliebte Touristenziele. Während manche Pinguinarten sich an die Anwesenheit von Menschen gewöhnen, zeigen andere negative Reaktionen in Bezug auf Störungen. Wir untersuchten die Auswirkungen von Tourismus auf Magellanpinguine (*Spheniscus magellanicus*) in der San Lorenzo Kolonie auf der Halbinsel Valdes, Chubut in Argentinien. Dabei wurden Parameter zum Verhalten, Physiologie und Reproduktion von brütenden Vögeln in von Touristen besuchten und nicht besuchten Gebieten erfasst. San Lorenzo wird seit 11 Jahren von Touristen in geringer Intensität besucht ($\sim$10.000 Besucher jährlich). Nach 15 Minuten eines simulierten Besuchs zeigten die Pinguine weniger wechselnde Kopfbewegungen und niedrigere Plasmawerte des Stresshormons Kortikosteron als Pinguine in nicht

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besuchten Gebieten. Allerdings zeigten die Pinguine in beiden Gebieten gleiche Basiswerte von Kortikosteron und auch ähnliche Werte erhöhten Kortikosteronspiegels nach einer akuten Stresssituation. Der Bruterfolg der Pinguine sowie das Kükenwachstum war ähnlich in beiden Gebieten. Wir vergleichen diese Ergebnisse mit bereits publizierten Resultaten aus Punta Tombo, eine seit mehr als 50 Jahren besuchte Kolonie mit über 120.000 Besuchern im Jahr. Einige (Reproduktion und Verhalten), aber nicht alle (Physiologie) Ergebnisse der untersuchten Parameter sind ähnlich. Dass ein physiologischer Unterschied besteht, könnte darauf hindeuten, dass der vorausgegangene Zeitraum der Besuche durch Touristen eine wichtige Rolle hinsichtlich der Reaktion der Vögel spielt. Daher ist eine Fortführung des Monitorings der Tourismusaktivitäten von Bedeutung, da die zeitliche Entwicklung der Störung durch Besucher Auswirkungen darauf hat, wie die Vögel auf die Besucher reagieren.

Introduction

Due to their perception as charismatic animals and the extensive attention they have received recently through films and other media, penguins and their wild breeding colonies are popular as ecotourism destinations. Humans have visited penguin colonies for decades, and currently millions of people visit penguin colonies all around the southern hemisphere (Boersma 2008). The ecotourism industry is often considered as an ecologically friendly and viable system providing for the conservation of resources, while also benefiting the economic structure of local communities (Brightsmith et al. 2008). Although ecotourism activities are typically initiated with the best intentions (but most of the time lacking biology-based management), penguins are potentially vulnerable to human activities (Ellenberg et al. 2006; Giese 1996).

Previous studies on penguins have shown variable effects of tourists visiting breeding colonies. With some exceptions, most of these studies report one or two parameters that could be affected by tourism, and do not present a more integrative analysis of tourism effects. Negative effects such as reduced breeding success, lower fledging weights, changes in normal behaviour, higher heart rates, lower nest densities, collapsed burrows, and higher stress-induced hormones in frequently visited sites have been observed in Humboldt Penguins (*Spheniscus humboldti*; Ellenberg et al. 2006), Yellow-eyed Penguins (*Megadyptes antipodes*; Ellenberg et al. 2007; McClung et al. 2004), Emperor Penguins (*Aptenodytes forsteri*; Burger and Gochfield 2007), and Magellanic Penguins (*Spheniscus magellanicus*) (Otley 2005). On the other hand, negative effects or no effects in breeding success and in the number of breeding pairs have been documented in Adélie Penguins (*Pygoscelis adelia*; Giese 1996; Woehler et al. 1994; Lynch et al. 2010) and Gentoo Penguins (*Pygoscelis papua*) in visited colonies (Cobley and Shears 1999; Trathan et al. 2008; Lynch et al. 2010; Otley 2005). These studies suggest that in some circumstances penguins can habituate to human visitation, but there is a wide variety of site-specific and time-related responses of the penguins to tourist visitation.

For Magellanic Penguins, most of the studies that have examined tourism disturbance effects have been from Punta Tombo (44°02′S, 65°11′W), the largest Magellanic Penguin colony in the world (Yorio and Boersma 1992; Walker et al. 2005, 2006; Fowler 1999). Tourists have been visiting Punta Tombo for more than 50 years (Boersma 2008), and currently over 120,000 people visit the colony annually (Organismo Provincial de Turismo, Chubut, Argentina, personal communication). Penguins at Punta Tombo appear to have habituated to human visitation (Walker et al. 2006; Yorio and Boersma 1992). In tourist areas at Punta Tombo, adult penguins first reacted to visitors at closer distances than in non-visited areas, and breeding success and fledgling weights were similar between areas (Yorio and Boersma 1992). While Fowler (1999) found that penguins that had been exposed to tourism did not respond to human presence as a stressor, he showed that penguins which had been exposed to some research visits over a few years—although not handled—did not show evidence of habituation. While Walker et al. (2006) found that baseline levels of the stress hormone corticosterone were the same in adult penguins living in visited and non-visited areas, adults nesting in tourist areas presented a reduced corticosterone stress response after 30 min of restraint. Whether this reduced response to an acute stressor is detrimental to penguins is still not known, but does represent a distinct physiological modification that is due explicitly to tourism disturbance.

The economic benefits of ecotourism encourage the opening of more penguin colonies to tourist visitation. One such colony is located on the northern tip of the Peninsula Valdes, Argentina, at the Estancia San Lorenzo (42°05′S, 63°49′W) where ecotourism started in 2000. At San Lorenzo, the number of visitors has been increasing since the opening of the colony. Currently, approximately 10,000 people visit annually (M. Machinea, personal communication).

In this study, we examine the effects of tourism on Magellanic Penguins through an integrated approach (i.e. behaviour, reproductive output and physiology) at the newly opened colony at San Lorenzo. We measured glucocorticoid stress hormones to quantify physiological differences between tourist-visited and non-visited penguins at San Lorenzo, and also measured a variety of behavioural

and reproductive parameters in visited and non-visited areas. Having quantified the effects of tourist visitation on the penguins at San Lorenzo, we then compared those patterns to those described in the previous studies at Punta Tombo. As such, we present an interesting comparison between a colony with a short history of visitation and with relatively low numbers of tourists to a colony with an extensive history of tourism and very high tourist numbers. We hypothesise that the previous history of tourist visitation of the colony may modify penguins' responses to tourism. We expect that some behavioural, reproductive and/or physiological parameters are affected differently in colonies with different tourist numbers and different lengths of time of tourist visitations.

Methods

Study area and species

We conducted the research in the Magellanic Penguin colony located at Estancia San Lorenzo, Peninsula Valdes, Chubut, Argentina (42°05′S, 63°49′W). The Peninsula Valdes has the status of a Natural Protected Area by the Argentine government and was declared a Human Heritage Site by UNESCO in 1999. In addition to penguins, Southern Right Whales (*Eubalaena australis*), Killer Whales (*Orcinus orca*), and Southern Elephant Seals (*Mirounga leonina*) attracted more than 300,000 tourists in 2008 (AANPPV 2010) to the stark and beautiful area. The penguin colony has approximately 55,000 breeding pairs (Schiavini et al. 2005), and received approximately 10,000 tourist during the most recent season (2009–2010). The colony was originally opened to tourist visitation in the year 2000. There is a delimited trail (803 m long) where people walk around to see the penguins, so the visitation is restricted to a very small area of the total colony. To do the research, we studied penguins nesting in the visited area and in a non-visited area of the colony during three reproductive seasons 2007–2008, 2008–2009, and 2009–2010. The non-visited area was similar to the visited area in breeding chronology, nest density, nest type, nest quality, and distance to the sea.

Magellanic Penguins have the conservation status of "near threatened" (IUCN 2010) because they are potentially vulnerable to anthropogenic activities such as oil spills or fishing (Skewgar et al. 2007; Garcia-Borboroglu et al. 2006) and climate change (Boersma 2008). Magellanic Penguins can live longer than 30 years (P.D. Boersma, personal communication). They start reproducing when they are 5–8 years old (Boersma 2008) and can reproduce at least 10 seasons (P.D. Boersma, personal communication). The reproductive season starts in September when male penguins arrive to the colony to rebuild their nests. Two to three weeks later, females arrive, and pairs start courtship and mating. Females lay two eggs. The incubation period last between 38 and 41 days, and is carried out by both members of the pair. Chicks start hatching around the second week of November. The brooding period lasts between 90 and 110 days. Chicks start fledging at the end of January. Adult penguins start moulting in February–March. Moult lasts up to 3 weeks, and birds fast during this time. At the end of March to early April, the penguins migrate to off-shore Uruguay and Brazil, and spend all winter foraging at sea (Yorio and Boersma 1994; Davis and Renner 2003).

Response to tourist visitation

During the 2009–2010 season, in both tourist-visited and non-visited areas, we simulated a tourist visit by walking around incubating female penguins for 15 min (see Walker et al. 2006 for exact methodology; $n = 12$ in each area). To determine if there were behavioural differences between visited and non-visited birds in response to this "visit", we counted alternate head turns (a defensive behaviour exhibited by *Spheniscus* penguins; Eggleton and Siegfried 1977; Ellenberg et al. 2006; Yorio and Boersma 1992) during four different 1-min intervals distributed through the 15-min visit. At the end of the 15 min, we caught the bird and took a blood sample to determine plasma corticosterone levels secreted in response to the visit. We used venipunture on the interdigitary (foot webbing) vein to collect the blood samples (75–150 µl) into heparinised microcapillary tubes. Then, the penguins were weighed with a spring scale (Pesola nearest 100 g) and bill length (BL), bill depth (BD), flipper length (FL), and foot length (FT) measured with dial callipers (nearest 0.1 mm) or a ruler (nearest 1 mm). Body size was calculated as:

$$\text{Body size} = 0.899(\text{BL}) + 0.924(\text{BD}) + 0.867(\text{FL}) + 0.907(\text{FT})$$

Coefficients were scores from a principal components analysis of standardised morphometric measurements by Hood et al. (1998). We did a regression analysis between body mass and body size. We used the subsequent residuals as a measure of body condition.

Acute stress response

After quantifying how the two groups of penguins responded to tourist visitation, we next wanted to quantify the baseline corticosterone levels (as high levels may be an indication of chronic stress) as well as the ability for penguins to respond to a novel stressor (i.e. capture and handling). During the incubation period of the 2008–2009 season, we sampled 23 birds exposed to tourism (13 males

and 10 females) and 22 not exposed to tourism (12 males and 10 females).

During incubation, we chose our study nests by searching the colony for females that were sitting on eggs. All nests were chosen so that birds sitting in the nest cup could not see the researcher approach until the person was <5 m away. Once located, nests were marked and checked daily until the male returned. When he returned (easily determined by seeing a new, clean, fat bird in the nest), we sampled the male on that or the next day, so the bird saw us at most only once before blood collecting. We chose females for the study in the same way, by marking incubating males and waiting for the clean, fat female to return from foraging at sea.

Once we captured the bird, its head was covered by an opaque cotton bag and then restrained in the lap of a researcher. We followed the typical capture-stress protocol which is commonly used in field studies (Romero and Wikelski 2001; Wingfield et al. 1992; Cockrem 2007). Initial blood samples were all taken within 3 min of capture because in many species blood samples collected after 3 min from initial disturbance can have significantly increased corticosterone levels (Romero and Reed 2005). Then, we collected three successive samples at 10, 30, and 45 min, again using venipunture as described above.

For all blood sampling, capillary tubes were stored on ice for up to 6 h in the field and then centrifuged for 10 min to separate the plasma. We froze the plasma at −20°C and transferred it frozen to the laboratory for analysis. We collected all blood samples early in the day (before 1230 hours), to avoid any variation in the daily normal circulating levels of corticosterone. After collecting the blood samples, penguins were weighed and measured to determine their body condition as described above.

Corticosterone measurement

We measured corticosterone in plasma samples following a modified direct radioimmunoassay (RIA) format without chromatography (Wingfield and Farner 1975). Each sample was assayed in duplicate after extraction in 4 mL of freshly distilled dichloromethane. Within-assay variability was 3.5%, calculated as the average coefficient of variation between all of the duplicate samples in the assay.

Breeding success

We measured breeding success during the three seasons of the study in both tourist-visited ($n = 50$, 44 and 44 nests, respectively) and non-visited areas ($n = 49$, 44, and 49 nests, respectively) of the colony. We recorded the number of eggs laid, the number of eggs hatched, and the number of chicks that were alive at the fifth week post-hatch. We checked the nests five times during the season and we did not touch any adult or chick to avoid the added stress of handling disturbance. We used the fifth week post-hatch as our last observation date, because after this time, chicks gather into crèches and it is impossible to tell which chick is which.

Chick growth

We followed 30 nests in each area during 2007–2008, where we took weekly measurements of the chicks until they fledged. We recorded hatching date and measured mass, flipper and foot length. We only followed nestlings from 2-egg clutches.

Statistical analysis

We used Microsoft Office Excel 2003, Tinn-R 1.19.4.7 (Faria 2009), and R 2.9.1 (R Development Core Team 2009) for all statistical analysis. We also used the libraries: MASS, nnet, nlme, lattice, and multilevel for R (Bliese 2008; Pinheiro et al. 2009; Sarkar 2009; Venables and Ripley 2002). We used the Shapiro–Wilks test for testing normality, and the Bartlett test for examining homogeneity of variances. We carried out different analysis depending on the type of data.

For response to visitation data, we fitted a Generalised Linear Model (GLM) to compare the number of alternate head turns between locations. We used a negative binomial distribution for the response variable (head turns) to deal with overdispersion, and log as the link function. Differences in hormone levels after 15 min of visitation between visited and non-visited areas were compared by analysis of variance (ANOVA).

For the acute stress response data, we carried out a correlation analysis to investigate if there was a correlation between time of capture and baseline levels of corticosterone. Differences in the stress response between visited and non-visited sites were compared by an ANOVA. We compared baseline levels of corticosterone to determine whether there were any differences between visited and non-visited adult penguins before the capture and restraint. We also compared the total or "integrated" corticosterone during the stress protocol by using the arithmetic trapezoid rule to calculate the area under the glucocorticoid stress response curve for each penguin (Walker et al. 2005). Body mass, body size, and body condition between areas were compared by ANOVA.

For the breeding success data, we fitted a Multinomial Logit Model (Faraway 2006) to compare the number of eggs laid, number of eggs hatched, and number of chicks alive at the fifth week of age; both between areas, and among seasons. We used this method because the response

variable was discrete and could only take three values 0, 1 or 2 (eggs or chicks). We chose the best model by Likelihood Ratio Test (LRT) (Hilborn and Mangel 1997).

Finally, for chick growth data, we had observations of the same individuals through time. These observations were not independent and tend to be correlated (Lindstrom and Bates 1990). We used a nonlinear model for weight, and nonlinear Mixed Effects models (Pinheiro and Bates 2002) for flipper and foot length, to compare the growth of the chicks between areas. We fitted the Logistic Growth Model with four parameters (Crawley 2007). We treated the chick id as a random effect in the models.

$$\text{Model}: y = a + (b - a)/(1 + \exp^{(c-x)/d}),$$

where a is the horizontal asymptote on the left (for low values of x), b is the horizontal asymptote on the right (for high values of x), c is the value of x at the point of inflection of the curve, and d is a numeric scale parameter on the x axis determining the rapidity of ascent. Model selection was done by LRT.

Results

Response to tourist visitation

The results showed no differences in body mass ($F_{1,21} = 0.22$, $P = 0.64$), body size ($F_{1,21} = 0.12$, $P = 0.73$), or body condition between tourist-visited and non-visited areas ($F_{1,21} = 0.11$, $P = 0.74$) at San Lorenzo. Penguins nesting in the tourist-visited area had fewer alternate head turns than penguins living in the non-visited area ($\chi^2_2 = 85.192$, $P < 0.0001$) (Fig. 1). We further found a significant difference in the plasma corticosterone levels after 15-min of visitation between penguins in tourist-visited (mean = 1.86 ng/mL; SE = 0.25) and non-visited areas (mean = 5.46 ng/mL; SE = 1.10; $F_{1,11} = 5.28$, $P = 0.03$; data not shown).

Acute stress response

Penguins in tourist-visited and non-visited areas responded to the stress of capture, handling, and restraint (Fig. 2). There was no correlation between the time of capture and baseline levels of corticosterone ($r = 0.078$). Baseline (unstressed) corticosterone concentrations were indistinguishable between areas and sexes (two-way ANOVA, area term: $F_{1,41} = 1.22$, $P = 0.27$; sex term: $F_{1,41} = 1.48$, $P = 0.23$; Fig. 2). Integrated corticosterone levels were similar between areas (two-way ANOVA, area term: $F_{1,41} = 0.0006$, $P = 0.98$), but there were differences between sexes (sex term: $F_{1,41} = 5.68$, $P = 0.02$). Females showed higher integrated corticosterone levels than males.

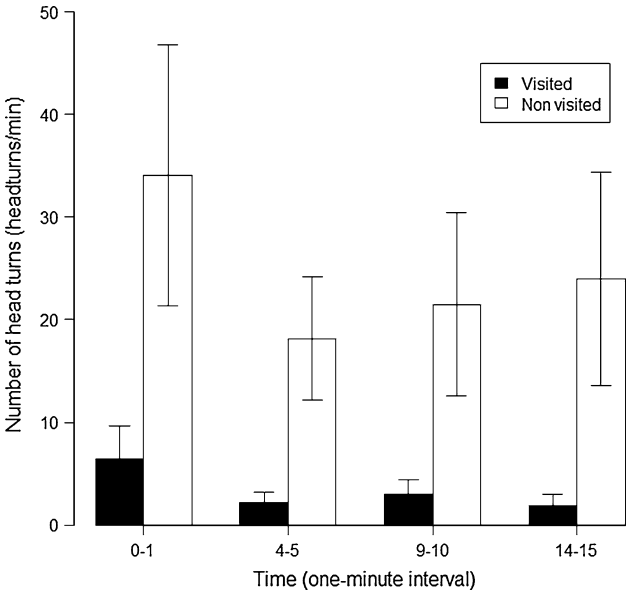

Fig. 1 Mean number of alternate head turns (in headturns/min, with SE bars) during four 1-min intervals (minute 0, 4, 9 and 14) during a 15-min simulated tourist visit to individual Magellanic Penguins (*Spheniscus magellanicus*) nests in both tourist-visited (*black bars*) and non-visited areas (*white bars*) of the San Lorenzo colony

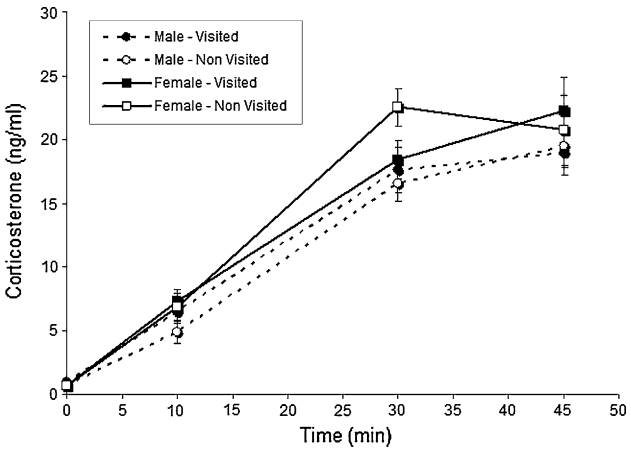

Fig. 2 Average baseline (time 0) and increases in corticosterone concentrations (with SE *bars*) during capture and restraint for incubating males (*dashed lines with circles*) and females (*solid lines with squares*) Magellanic penguins, in tourist-visited (*black*) and non-visited (*white*) areas of the San Lorenzo colony

Body mass (Males: $F_{1,23} = 0.42$, $P = 0.52$; Females: $F_{1,18} = 0.11$, $P = 0.74$), body size (Males: $F_{1,23} = 1.6$, $P = 0.22$; Females: $F_{1,18} = 0.84$, $P = 0.37$), and body condition (Males: $F_{1,23} = 0.34$, $P = 0.56$; Females: $F_{1,18} = 0.13$, $P = 0.72$) were not different between areas.

Breeding success

We found no differences between areas or among seasons for eggs laid ($\chi^2_3 = 1.07$, $P = 0.78$), eggs hatched

Table 1 Number of nests, eggs laid, hatched chicks, and live chicks in tourist-visited and non-visited areas of the San Lorenzo Magellanic Magellanic Penguin (*Spheniscus magellanicus*) colony during three breeding seasons

Season	Tourist-visited area					Non-visited area				
	No. nests	Eggs laid	Eggs hatched	Chicks	Breeding success	No. nests	Eggs laid	Eggs hatched	Chicks	Breeding success
2007–2008	50	98	83	73	1.46 (0.79)	49	98	75	60	1.22 (0.82)
2008–2009	44	83	65	56	1.27 (0.82)	44	83	62	60	1.36 (0.87)
2009–2010	44	87	76	65	1.48 (0.7)	49	96	81	59	1.20 (0.89)

Breeding success was calculated as the number of chicks divided by the number of nests; the SD is in parentheses

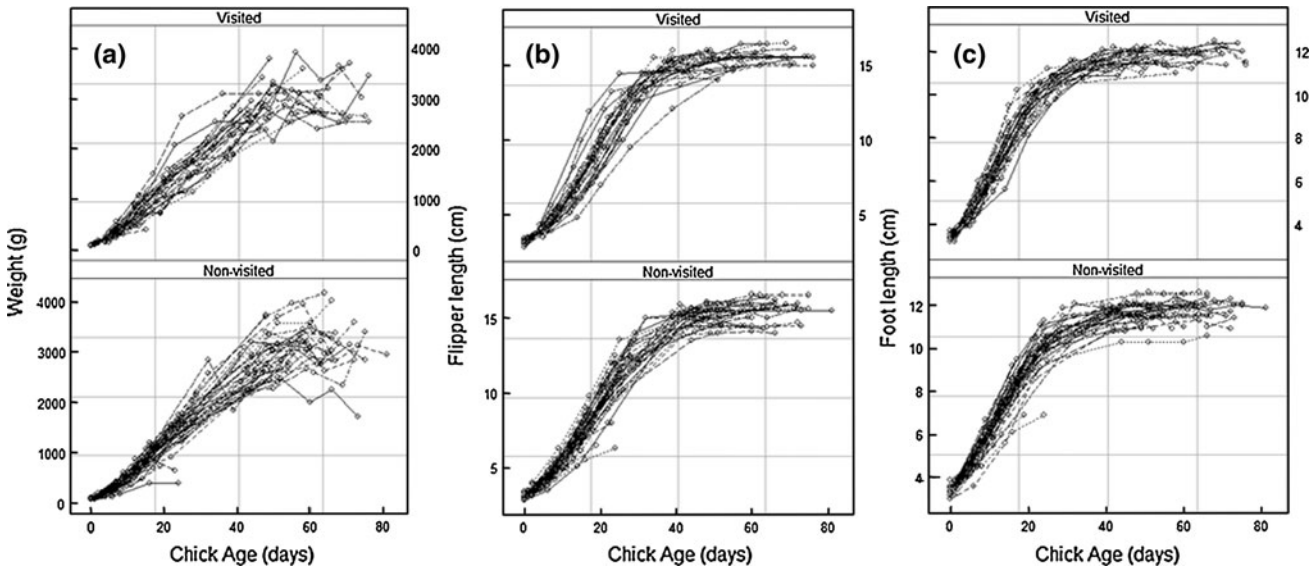

Fig. 3 **a** Weight (in g), **b** flipper and **c** foot length (in cm) at age (in days) of individual chicks nesting in the tourist-visited (*above*) and the non-visited (*below*) areas of the San Lorenzo colony

($\chi^2_6 = 3.65$, $P = 0.72$), or chick survival ($\chi^2_6 = 7.33$, $P = 0.29$) until week number five (Table 1).

Chick growth

Chick weights were similar between areas ($\chi^2_4 = 0.16$, $P = 0.99$) (Fig. 3). There was no differences in growth rate of flippers between tourist-visited and non-visited chicks ($\chi^2_4 = 8.17$, $P = 0.08$). For foot length, we found that there were differences between visited and non-visited chicks in the parameter d in the equation ($\chi^2_1 = 5.21$, $P = 0.02$), which was the scale parameter, and could be interpreted as a growth rate since it determines the rapidity of ascent in the curve. Finally, growth was different among individual chicks (random effects), indicating that the variation among individuals was important, and needs to be considered.

Discussion

Tourism effects at San Lorenzo

Ecotourism is perceived as a potentially effective way to preserve species and the habitat where they live, and also to bring economical benefits to the local community (Brightsmith et al. 2008). While some animals have shown tolerance to ecotourism, (i.e. waterbirds; Klein et al. 1995), others have not. For example, Hoatzin juveniles (*Opisthocomus hoazin*) showed reduced survival and stronger hormonal responses in disturbed areas (Müllner et al. 2004), and Wandering Albatrosses (*Diomedea exulans*) showed higher heart rates (Weimerskirch et al. 2002) in disturbed versus non-disturbed areas. While the presence of people did not displace wintering shorebirds from high quality habitats, their presence did reduce foraging rates

(Yasué et al. 2008). As summarized in the "Introduction", in penguins, tourist visitation has generated different effects depending on the colony or species studied. Some examples are: Humboldt Penguins showed increased heart rates, lower nest densities and lower reproductive success at frequently visited sites (Ellenberg et al. 2006); and Gentoo Penguins showed no differences in breeding success in a tourist-visited colony (Otley 2005) and lower breeding success in other frequently visited colonies (Lynch et al. 2010).

In our study, Magellanic Penguins at San Lorenzo—a colony with 11 years of tourist visitation—showed no obvious negative consequences of the visitation. When subjected to a simulated tourist visit, both a behavioural response (alternate head turns) and a physiological response (glucocorticoid hormone levels) were lower in birds nesting in tourist-visited areas compared to non-visited areas. This suggests that habituation—both behavioural and physiological—is occurring in tourist-visited birds.

We also found that the baseline corticosterone level was similar for penguins living in tourist-visited and non-visited areas at San Lorenzo. This suggests that penguins in the tourist area are not under chronic stress. Furthermore, the integrated corticosterone concentration after the capture stress protocol was also similar between areas. Thus, both groups of birds are capable of a robust stress response to a novel stressor. Body mass, body size and body condition of sampled birds were similar between areas.

While there were differences between males and females in the integrated corticosterone levels, we have no good explanation why this may be the case. Low body condition has been associated with higher corticosterone levels (Kitaysky et al. 1999; Hood et al. 1998). We did not find differences in body condition between areas in male and female Magellanic Penguins, so we cannot attribute the higher levels in integrated secreted corticosterone to a bad body condition in females. Further examination of sex-based differences in stress physiology may be warranted, particularly in periods, such as egg laying, when physiological processes are very different between the sexes.

In addition to behavioural and physiological similarities between tourist-visited and non-visited birds, breeding success was also similar for Magellanic Penguins nesting in the two areas of the colony. We also found no differences in the majority of growth patterns in chicks living in both areas with the exception of chicks in the tourist-visited area having higher foot growth rates than in the non-visited area (*d* parameter in the model). This one "significant" difference actually represents a real-time difference of only 8 h. As such, while statistically significant, the biological significance of this difference is likely irrelevant.

A comparison of colonies

As summarized in Table 2, in many instances, the patterns that we observed at San Lorenzo—a colony with a shorter history of visitation and a much lower number of annual visitors—are similar to patterns observed at Punta Tombo—a colony with more than 50 years of visitation (Boersma 2008) and with current tourist numbers at well over 120,000 annually. Previous studies from Punta Tombo showed that both alternate head turns and plasma corticosterone concentration after 15 min of a standardised

Table 2 A comparison of penguin responses to tourist visitation at San Lorenzo and Punta Tombo colonies

Response	San Lorenzo[a] Tourist-visited versus non-visited comparison	Punta Tombo Tourist-visited versus non-visited comparison
Body mass	Similar	Similar[b,c]
Body condition	Similar	Similar[b]
No. of head turns	Higher in non-tourist area	Higher in non-tourist area[b,c]
Cort after $5^3/15^{1,4}$ min of visitation	Higher in non-tourist area	Higher in non-tourist area[b,c]
Acute stress		
Baseline cort	Similar	Similar[b]
Integrated cort	Similar	Lower in tourist area[b]
Breeding success		
Eggs laid	Similar	Similar[d]
Chicks hatched	Similar	No data
Live chicks	Similar	Similar[d]
Chick growth		
Flipper length	Similar	No data
Foot length	Similar	No data
Weight	Similar	Similar[d]

[a] This study
[b] Walker et al. (2006)
[c] Fowler (1999)
[d] Yorio and Boersma (1992)

"visit" were lower in penguins nesting in the tourist area than in penguins nesting away from the tourist trail (Walker et al. 2006), similar to those observed in this study. There were also no differences in baseline levels of corticosterone between areas, but again, differences in corticosterone concentrations between sexes were found (Hood et al. 1998), similar to our findings. In general, these findings suggest that, at both colonies, penguins have habituated to having tourist visitations, regardless of the history of visitation or current tourist numbers.

Interestingly, penguins nesting in the tourist trail at Punta Tombo showed a decreased glucocorticoid stress response to a novel stressor (i.e. capture) compared to penguins nesting in undisturbed areas (Walker et al. 2006). We did not find this difference in response to novel stressors in penguins in visited and non-visited areas at San Lorenzo (see Table 2). Whether this reduced stress response to novel stressors in tourist-exposed adult penguins at Punta Tombo is beneficial or detrimental is unknown. However, the same pattern has been observed in two other instances in species living in more disturbed versus undisturbed environments: Marine Iguanas (*Amblyrhynchus cristatus*) in tourist versus non-tourist areas in the Galápagos (Romero and Wikelski 2002), and in city-born versus rural-born European Blackbirds (*Turdus merula*; Partecke et al. 2006). It could be hypothesised that a lower stress response in these disturbed individuals may allow them to avoid the negative consequences of repeated elevated glucocorticoids when responding to stressors (Wingfield 1994; Johnson et al. 1992). If this is a benefit for animals living in highly disturbed environments, the mechanism/development of this "benefit" has not yet occurred for penguins nesting in disturbed tourist areas of San Lorenzo.

In examining what might be causing the reduced response to novel stressors in tourist-visited penguins at Punta Tombo, Walker et al. (2006) showed that the lower response was due to an actual physiological difference in the capacity of the adrenal glands to secrete corticosterone. While not explicitly tested, since San Lorenzo penguins responded similarly to a novel stressor regardless of location, it is unlikely that any modification of adrenal function is present in tourist-area penguins at the newly opened colony.

In contrast to the potential benefit of not expressing a robust stress response to novel stressors, it may also be hypothesized that the decreased capability to secrete glucocorticoids from the adrenal gland could be detrimental in instances when these animals need to utilize such hormones. In times when a robust stress response is warranted, a "normally" functioning adrenal gland would likely be beneficial. We have no evidence at either Punta Tombo (with modified adrenals) or San Lorenzo (normal adrenal response) that penguins living in tourist areas are more susceptible to other types of stressors in their lives. Indeed, over the short term, or in the first years of life, this reduced functionality of adrenals might not be problematic. However, over a lifetime, a continual decreased ability to deal adequately with stressors may ultimately "add up" in the end—a condition that tourist-visited penguins at Punta Tombo may be experiencing. If the hypothesis that many years of reduced adrenal capability may "get you in the end", a study more than 30 years long is required to document such "senescent" possibilities.

The interpretation that habituation is occurring in Magellanic Penguins at Punta Tombo—and now at San Lorenzo—has generated some discussion. Cyr and Romero (2009) have stated that penguins at Punta Tombo are under "chronic stress", due to the reduced adrenal function. As we stated above, the implications of such physiological alterations are not yet well documented in wild populations since it is difficult to translate how they can be measured in terms of population viability. Regardless of the negative implication given to describing chronic stress in Magellanic Penguins at Punta Tombo (Cyr and Romero 2009), it is interesting that, in the colony at San Lorenzo, by Cyr and Romero's definition, there is no chronic stress suffered by penguins in tourist-visited areas.

Finally, in one last discussion relating to chronic stress in Magellanic Penguins at Punta Tombo, Cyr and Romero (2009) stated that as evidence of chronic stress (as hypothesised for those penguins), negative population, behavioural, or reproductive characteristics must also be measurable. In both Punta Tombo and San Lorenzo, there is no evidence of decreased reproductive rate, survival or chick growth associated with human disturbance. Thus, an important component of what was determined as "chronic stress" (Cyr and Romero 2009) is not present in the penguins from either San Lorenzo or Punta Tombo.

The conundrum of how and why Magellanic Penguins in tourist areas at Punta Tombo have come to have a reduced adrenal function is a mystery. Is it the long history of visitation? Or rather the currently high levels of tourist visitation? To disentangle this mechanism, an interesting future study would be to measure the physiological response of penguins in a colony with a long-term history of human visitation (as in Punta Tombo) but low levels of tourists (i.e. San Lorenzo). Alternatively, a new colony could be opened to tourism with a very high level of initial exposure. While the former situation may already be found in Argentina [for example, the colonies at Cabo dos Bahias (47°06′S, 65°52′W) or Cabo Virgenes (52°22′S, 68°24′W)] and thus tested relatively easily, the potential risk of a "hit them hard" experimental strategy of the second option is likely a proposition of concern.

In summary, we were presented with a unique opportunity to compare a single scenario—tourist visitation to

Magellanic Penguin colonies—in two very different contexts: long history versus short history of tourist visitation and high tourist numbers versus lower numbers. We found, in fact, that the historical context does seem to matter, but the interpretation of the effects is complex. While penguins in both colonies seem to be "doing well", as measured by reproductive success, chick survival and growth, and stress hormones, perhaps the longer-term disturbance at Punta Tombo is causing some larger-scale modification that we cannot presently detect. As our results suggest that different colonies of the same species may be responding to current tourism levels in different ways, the monitoring of potential negative effects in any visited colony should be continued. In addition, the control of visitation is also very important because negative effects may be seen after a delay, and the tourist scenario is changing rapidly in the region. At best, our results from San Lorenzo, compared to the previous results from Punta Tombo, suggest that a gradually increasing intensity of human visitation is better than high intensities of tourists in order for the penguins to cope with the disturbance. Ultimately, it is important to note that, in species with long and complex life histories, long-term monitoring studies may be the only way to ever accurately determine the true costs associated with human disturbance patterns.

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